A Lung Retention Model Based on Michaelis–Menten-like Kinetics

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A Michaelis–Menten (MM)-like kinetic model for pulmonary clearance and retention of insoluble dusts was developed and validated by comparing our predictions with experimental data from F344 rats. Published data from inhalation studies involving accumulation and elimination of photocopy test toner, antimony trioxide, carbon black, and diesel exhaust particles were investigated. Numerical integration techniques were used to solve mass balance relationships based upon dust retention in a single lung compartment and clearance via an MM-like kinetic process. The model fit most of the experimental data well. The parameters of MM-like clearance kinetics, which had been derived strictly from the elimination phase, accurately predicted dust retention during the elimination as well as accumulation phases. Furthermore, parameters estimated from one study could accurately predict retention of the same dust in other studies. Particle density and gender of the animals had no effect on the goodness of fit of model predictions. This study suggests that MM-like kinetics offer a reasonable description of particle clearance from the pulmonary region of the rat lung that is more parsimonious than existing particle-clearance models and therefore more suitable for use with small amounts of data. *Key words:* insoluble dusts, Michaelis–Menten kinetics, pulmonary, retention model. *Environ Health Perspect* 105:496–503 (1997).

Lung clearance and retention of spherical particles was substantially quantified by the comprehensive model published by the Task Group on Lung Dynamics in 1966 (*1*). The model postulated that alveolar clearance involved multicompartmental linear processes. In the ensuing three decades, these underlying assumptions have been challenged by numerous investigations that point to nonlinear behavior of clearance as a function of lung burden. In particular, recent models of pulmonary retention and clearance of dusts have focused upon three nonlinear processes, namely, sequestration, overloading, and redistribution.

Sequestration, first recognized by Soderholm (*2*), describes a phenomenon whereby the alveolar clearance of very large lung burdens essentially ceases. This concept has been used to explain the clearance and retention behavior of diesel exhaust particles (DEP) (*3–5*), carbon black (CB) (*6*), quartz (*7*), titanium dioxide (7), photocopy test toner (PTT) (*7*), and amosite fibers (*8*). However, because the characteristics of particle sequestration have not been fully elucidated, various types of models have been used to account for the phenomenon. For example, some investigations considered sequestration to be a process in which clearance ceased completely (*3,4,8–11*), while others regarded clearance as being merely very slow (*5–7,12*), or not applicable in cases involving low exposure (*13*).

Dust overloading, hypothesized first by Bolton et al. (*14*) and subsequently by Morrow (*15,16*), is a phenomenon in which the lung maintains its normal clearance rate until the burden reaches a threshold, whereupon clearance progressively slows. This phenomenon is based upon empirical observations that, above such a threshold, the alveolar clearance of exposed animals becomes significantly slower than those of control groups. As a result, compartmental models have included threshold values that defined the transition from normal to overloading conditions (*5,9,10*).

Finally, particle redistribution is a dynamic feedback process in which phagocytized particles are released from dying alveolar macrophages (AMs) into the alveolar space where they are rephagocytized by newly recruited AMs. This feedback process has been demonstrated in various experiments (*17–19*) and has been incorporated into recent retention models (*20–26*).

Because clearance and retention models, which explicitly account for sequestration, overloading, and/or redistribution, have become increasingly complex, we propose herein an alternative nonlinear model based upon Michaelis–Menten (MM)-like kinetics. This model is consistent with the treatment by Smith (*12*), who implicitly recognized the appropriateness of MM-like kinetics for alveolar clearance. We previously presented the rationale underlying the clearance portion of this model and developed a linear relationship between pulmonary clearance half-time and lung burdens to evaluate the fit of the model to published data of PTT, antimony trioxide (Sb_2O_3), DEP, and polyvinyl chloride powder (*27*). To further validate the full model (including both accumulation and elimination phases), we tested the model predictions of the temporal behavior of lung burdens, which were experimentally determined in a variety of inhalation studies in F344 rats.

Materials and Methods

Materials. We selected published data involving lung burdens measured over time in F344 rats exposed to Sb_2O_3 (*29*), PTT (*8,30*), CB (*6*), and DEP (*4,5,31*) by inhalation at various concentrations in either subchronic (12–18-week) or chronic (24-month) studies. Table 1 summarizes the protocols of these inhalation experiments. Note that some investigations studied only dust accumulation (*4,8*), some only elimination (*6,31*), and others both accumulation and elimination (*5,29,30*). These studies involved particles of both large diameters [PTT, mass median aerodynamic diameter (MMAD)= 4 μm; Sb_2O_3, MMAD = 3.5 μm] and small diameters (DEP, MMAD = 0.19–0.25 μm; CB, MMAD = 0.24 μm), as well as dusts of various densities (5.2 g/cm^3 for Sb_2O_3, 2 g/cm^3 for DEP and CB, and 1.2 g/cm^3 for PTT).

Lung retention model. Insoluble particles are deposited in various portions of the rat lung and cleared by several mechanisms. Relatively large particles are likely to be deposited in the conducting airways and are then removed rapidly (over a period of hours to a few days) by mucociliary clearance. Smaller particles tend to be deposited in the alveoli and are then cleared predominately by AMs, which phagocytize the particles and transport them to the ciliated epithelium. A secondary mechanism for clearance in the deep lung involves transport to lung-associated lymph nodes, possibly mediated by AMs or via direct penetration of particles through the interstitium.

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Because these clearance mechanisms in the deep lung are relatively slow (working over a period of weeks to months), the vast majority of the total lung burden resides in the alveolar region following chronic exposure to insoluble dusts. Thus, our model treated the rat lung as a single compartment consisting exclusively of the pulmonary region (the alveoli and the supporting structures) and ignored a separate compartment for the tracheobronchial region, which contributes relatively little to the total lung burden (*4*). Experimental data reporting burdens of dusts in lung-associated lymph nodes were not used for testing our model.

In keeping with our earlier treatment of MM-like clearance (*27*), we defined the pulmonary clearance rate coefficient $k = \ln(2)/T_{1/2}$, where $T_{1/2}$ is the clearance half-time for each dust, according to the following relationship:

$$k = \frac{k_{max} \cdot m_{1/2}}{m_{1/2} + m} \quad (1)$$

where k_{max} represents the maximum rate coefficient of pulmonary clearance, $m_{1/2}$ represents the characteristic burden at which $k = 1/2 \cdot k_{max}$, and m represents the lung burden at which k is evaluated.

During the accumulation phase, when rats are continuously exposed to dust according to a particular exposure regimen [e.g., 30 hr/week for 13 weeks in the study by Newton et al. (*29*)], particles accumulate in the lung during the period of daily exposure (e.g., 6 hr), and the mass balance governing the lung burden is given by

$$\frac{dm}{dt} = V_t \cdot E_A \cdot x - k \cdot m \quad (2)$$

where V_t is the ventilation rate (a function of the animal's age), E_A is the particle deposition efficiency, and x is the exposure concentration. Immediately following daily exposure, the lung reaches the initial postexposure burden, designated m_0. During the period prior to the next daily exposure (e.g., 18 hr), the burden is cleared according to the following relationship:

$$\frac{dm}{dt} = -k \cdot m \quad (3)$$

where

$$k = \frac{k_{max} \cdot m_{1/2}}{m_{1/2} + m_0}. \quad (1')$$

Table 1. Inhalation experiments involving measurements of lung burden over time in F344 rats

Dust	MMAD (μm)	Specific density	Sex	Phase of study	Exposure concentration (mg/m^3)	Exposure regimen	Reference
Sb_2O_3	3.5	5.2	M & F[a]	Accumulation	0.25, 1.08, 4.92, 23.46	30 hr/week for 13 weeks	(*29*)
				Elimination	NE	–	(*29*)
PTT	4	1.2	M & F[a]	Accumulation	1, 4, 16.1, 63.2	30 hr/week for 90 days	(*30*)
				Elimination	NE	–	(*30*)
PTT	4	1.2	M & F[a]	Accumulation	1, 4, 16	30 hr/week for 24 months	(*9*)
CB[b]	0.24	2	M	Elimination	6.4, 7.1, 6.7[c]	140 hr/week for 12 weeks[c]	(*6*)
DEP	0.21	2	M & F[a]	Elimination	0.15, 0.94, 4.1[c]	35 hr/week for 18 weeks[c]	(*31*)
DEP[d]	0.19	2	M	Accumulation	5.91	140 hr/week for 12 weeks	(*5*)
				Elimination	NE		(*5*)
DEP	0.25	2	M & F[a]	Accumulation	0.353, 3.47, 7.08	35 hr/week for 24 months	(*4*)

Abbreviations: MMAD, mass median aerodynamic diameter; Sb_2O_3, antimony trioxide; M, males; F, females; NE, no exposure; PTT, photocopy test toner; CB, carbon black; DEP, diesel exhaust partiulate.
[a]Combination of both males and females.
[b]Agglomerated particles (primary size = 0.07 μm).
[c]Concentrations and regimen were used to build up lung burdens.
[d]Agglomerated particles (primary size = 0.04 μm).

Equations 3 and 1′ are also applicable during the weekends and after termination of exposure; the latter will be referred to hereafter as the elimination phase. Note that when particles are not deposited in the lung, k (in Equation 1′) is a function of m_0 and is independent of the postexposure time. Values of m were estimated during the accumulation and elimination phases by numerically integrating Equations 2 and 3, as described in Appendix A.

Estimation of model parameters. The parameters to be estimated under the model, defined by mass balance Equations 2 and 3, are V_t, E_A, k_{max}, and $m_{1/2}$. Because V_t depends on the ages of the animals and, in part, on the particular laboratory where each inhalation study was conducted, we attempted to match the method of estimation to the published data as closely as possible. For the study by Strom et al. (*5*), we used the following function reported by the same authors (*6*) for male F344 rats:

$$V_t\,(\text{ml/min}) = 278e^{-\ln(2)(93)/t}, \quad (4)$$

where t is the animal's age in days. Likewise, for the later study from the same laboratory (*6*), we used a similar relationship reported by the authors:

$$V_t\,(\text{ml/min}) = V_M e^{-\ln(2)(50)/t} \quad (5)$$

where V_M represents the maximum ventilation rate. Since postexposure ventilation rates were reported to be 117, 123, and 154 ml/min among animals of the 1-week, 3-week, and 41-day groups, we adjusted V_M to values of 217, 202, and 227 ml/min, respectively, so that postexposure ventilation rates would correspond exactly to the reported values. In other studies, ventilation rates were not reported, so we used the published relationship of Guyton (*32*) between V_t and body weight (bw) in grams, at age t in days:

$$V_t\,(\text{ml/min}) = (2.1)(\text{bw})^{0.75}. \quad (6)$$

For the study by Newton et al. (*29*), body weight was estimated for male F344 rats from the following relationship given by Strom et al. (*5*) because it corresponded well to the published body weight curves of Newton et al. (*29*):

$$\text{bw (g)} = 460e^{-\ln(2)(104)/t}. \quad (7)$$

For female rats in the same study (*29*), the body weight from Equation 7 was adjusted by a factor of 84.5%, representing the estimated ratio of the average body weight of 7-week-old female rats to the corresponding males. Finally, for the studies by Wolff et al. (*4*), Bellmann et al. (*9*), Muhle et al. (*30*), and Griffis et al. (*31*), we estimated body weights from the following relationships published by Bellmann et al. (*8*):

$$\text{bw (g)} = 143 + (46.6)\ln(t) \quad (8)$$

for male rats, and

$$\text{bw (g)} = 38 + (42.5)\ln(t) \quad (9)$$

for female rats.

The MM-like parameters were estimated from the following relationships given by Yu and Rappaport (*27*):

Table 2. Particle deposition efficiencies used to fit Michaelis–Menten (MM)-like retention models

Dust	Range of particle deposition efficiency (%)	Source of data used to fit the MM-like retention model
Sb_2O_3	4.1–8.8	Newton et al. (*29*)
PTT	2.5–5.7	Muhle et al. (*30*)
PTT	2.8–4.1	Bellmann et al. (*9*)
CB	11–18	Strom et al. (*6*)
DEP	8–12	Griffis et al. (*31*)
DEP	11–15	Wolff et al. (*4*)
DEP	28–30	Strom et al. (*5*)

Abbreviations: Sb_2O_3 antimony trioxide; PTT, photocopy test toner; CB, carbon black; DEP, diesel exhaust particulate.

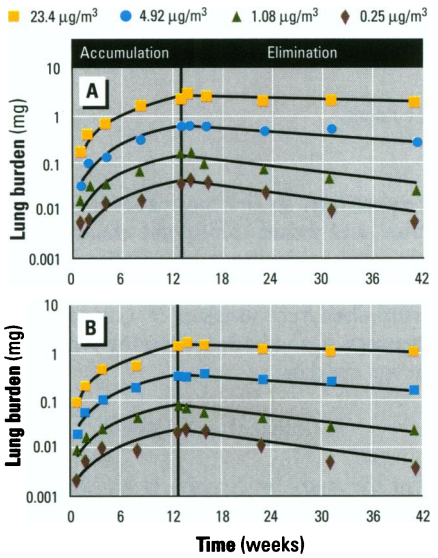

Figure 1. Accumulation and elimination of Sb_2O_3 in F344 male (A) and female (B) rats in a subchronic study by Newton et al. (*29*). Solid lines represent model predictions; symbols represent experimental data. (The Michaelis–Menten-like clearance parameters for the model were estimated from the elimination phase of the same study.)

$$k_{max} = \ln(2)/\alpha \quad (10)$$

and

$$m_{1/2} = \alpha/\beta \quad (11)$$

where α (common to all dusts) and β (specific to each dust) are, respectively, the estimated values of the intercept and slope of the linear relationship between $T_{1/2}$ and lung burden. The rationale for development of this linear relationship was discussed previously (*27*). From Equations 10 and 11, k_{max} was estimated to be 0.009/day for F344 rats (*27*) and $m_{1/2}$ was estimated to be 0.69 mg for Sb_2O_3 [reported by Yu and Rappaport (*27*) from data of Newton et al. (*29*)], 0.97 mg for PTT [reported by Yu and Rappaport (*27*) from data of Muhle et al. (*30*)], 2.49 mg for DEP [reported by Yu and Rappaport (*27*) from data of Griffis et al. (*31*)], and 1.11 mg for CB [estimated in this study from data of Strom et al. (*6*)]. Note that values of $m_{1/2}$ were derived from data obtained exclusively from the elimination phase of each experiment and that for PTT and DEP, values of $m_{1/2}$ that were estimated from one study were applied to other studies involving the same dust.

Finally, E_A was determined empirically for each experimental data set [by experimental group, dust, exposure concentration, duration (if applicable), and gender (if applicable)] by fitting Equations 2 and 3 to the data after including all other terms in the model. Table 2 shows the estimated ranges of E_A for each of the data sets for the various experimental groups.

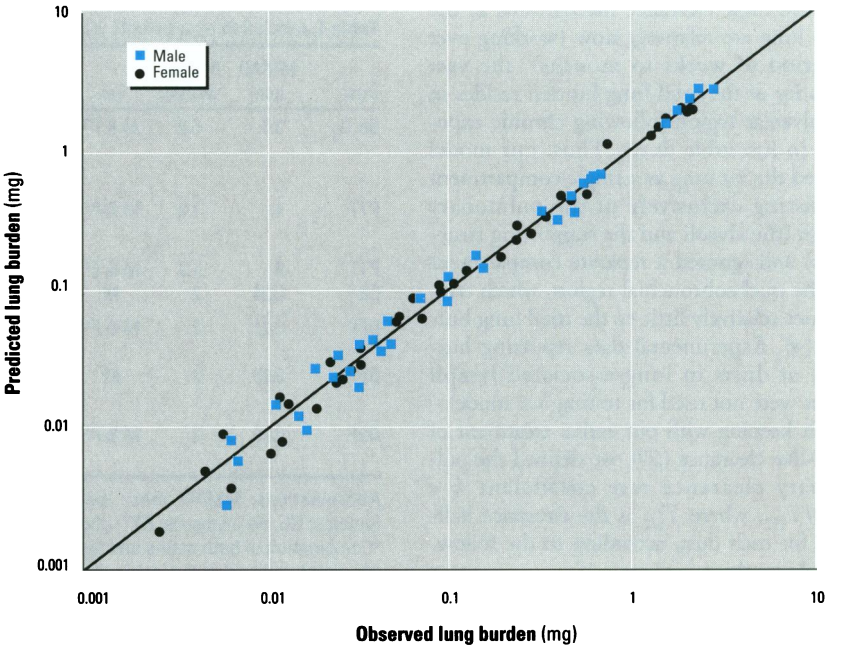

Figure 2. Overall comparison of model predictions and experimental data of Sb_2O_3 observed by Newton et al. (*29*).

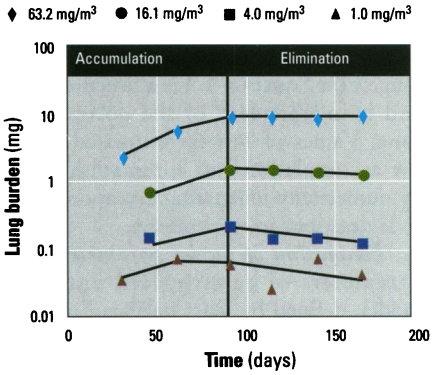

Figure 3. Accumulation and elimination of photocopy test toner in F344 female rats exposed for 90 days in a subchronic study by Muhle et al. (*30*). Solid lines represent model predictions; symbols represent experimental observations. (The Michaelis–Menten-like clearance parameters for the model were estimated from the elimination phase of the same study.)

Results

Antimony trioxide. Newton et al. (*29*) investigated both accumulation (for 13 weeks) and elimination (for an additional 28 weeks) of Sb_2O_3 in rats exposed to four exposure concentrations in a subchronic inhalation study. Figure 1 shows accumulation and elimination of the dust over time in male (Fig. 1A) and female (Fig. 1B) rats using values of k_{max} and $m_{1/2}$ that were estimated from the elimination phase of the same study. The model predicted the behavior of all experimental data quite well. Furthermore, the residuals were found to be unremarkable with no apparent gender effect on model predictions (Fig. 2).

Photocopy test toner. Muhle et al. (*30*) investigated PTT accumulation (up to 90 days) and elimination (for an additional 75 days), whereas Bellmann et al. (*8*) examined only accumulation of PTT (for 24 months). Figure 3 depicts the predicted and observed burdens in female rats exposed to different dust concentrations, Figure 4 shows the burdens in males exposed to various concentrations of PTT in the study by Muhle et al. (*30*), and Figure 5 shows the results from the study by Bellmann et al. (*8*). In each case, k_{max} and $m_{1/2}$ were estimated from the elimination phase of the study by Muhle et al. (*30*). These results indicate good fits of all data to the model, with the possible exception of the data from Muhle et al. (*30*) at

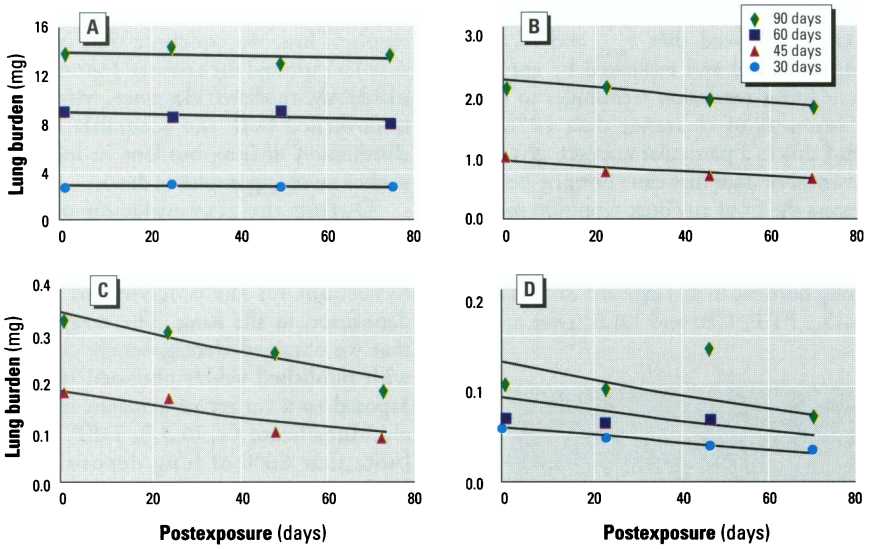

Figure 4. Elimination of photocopy test toner in F344 male rats exposed to 63.2 mg/m^3 (A), 16.1 mg/m^3 (B), 4.0 mg/m^3 (C), and 1.0 mg/m^3 (D) in a subchronic study by Muhle et al. (*30*). Solid lines represent model predictions; symbols represent experimental observations. (The Michaelis–Menten-like clearance parameters for the model were estimated from the elimination phase of the same study.)

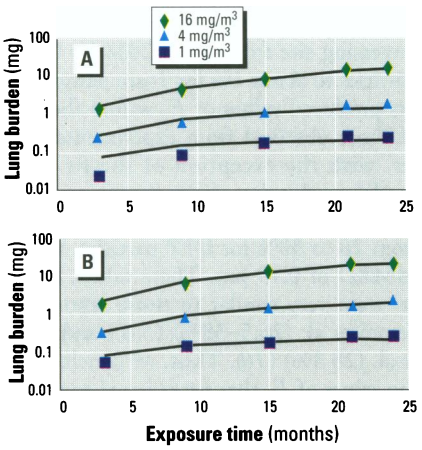

Figure 5. Accumulation of photocopy test toner in F344 female (A) and male (B) rats in a chronic study by Bellmann et al. (*9*). Solid lines represent model predictions; symbols represent experimental data. [The Michaelis–Menten-like clearance parameters for the model were estimated from the elimination phase of the study by Muhle et al. (*30*).]

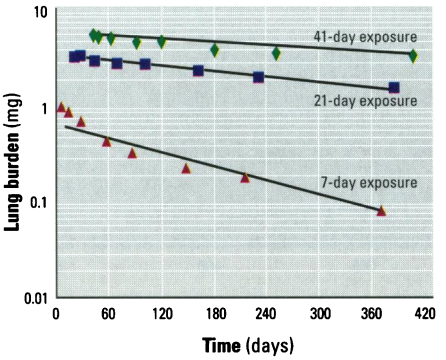

Figure 6. Experimental observations (symbols) on the elimination of carbon black from the lungs of F344 male rats in a study by Strom et al. (*6*). Solid lines represent model predictions. (The Michaelis– Menten-like clearance parameters for the model were estimated from the elimination phase of the same study.)

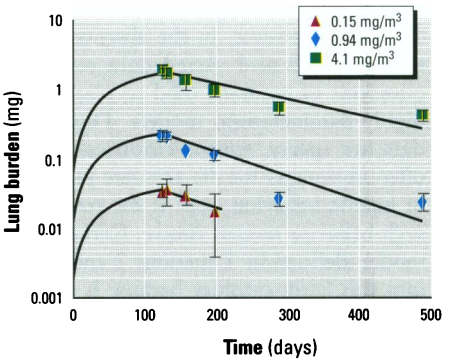

Figure 7. Experimental observations (mean ± SD) on the elimination of diesel exhaust particulate from the lungs of F344 rats in a study by Griffis et al. (*31*). Solid lines represent model predictions. (The Michaelis– Menten-like clearance parameters for the model were estimated from the elimination phase of the same study.)

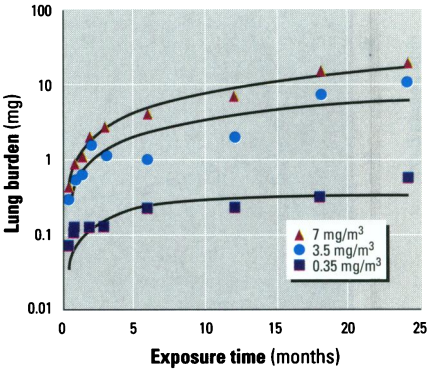

Figure 8. Accumulation of diesel exhaust particulate in F344 rats in a chronic study by Wolff et al. (*4*). Solid lines represent model predictions; symbols represent experimental observations. [The Michaelis–Menten-like clearance parameters for the model were estimated from the elimination phase of the study by Griffis et al. (*31*).]

the lowest concentration (Fig. 4D). Again, no effect on model fit by gender was observed (data not shown). The model, given the k_{max} and $m_{1/2}$ estimated from the elimination phase, accurately predicted the burdens during the accumulation and elimination phases in the same study by Muhle et al. (*30*). Furthermore, the parameters estimated from the study by Muhle et al. (*30*) accurately predicted lung burdens during the accumulation phase of the study by Vincent et al. (*8*), suggesting that the MM-like clearance parameters were valid between phases and between studies.

Carbon black. Strom et al. (*6*) investigated elimination of CB for 1 year following accumulation of a range of burdens in male F344 rats exposed at about 7 mg/m^3 for 7–41 days. Results are shown in Figure 6, using values of k_{max} and $m_{1/2}$ that were estimated from the same study. The overall fit of the model was good, despite some underestimation of lung burdens at early stages (<60 days) of the experiment in which the animals were exposed for 7 days.

Diesel exhaust particle. Three studies involved inhalation of DEP: Griffis et al. (*31*) investigated only elimination; Wolff et al. (*4*) studied only accumulation; and Strom et al. (*5*) examined both accumulation and elimination. Figures 7–9 depict these data and the corresponding model predictions where values of k_{max} and $m_{1/2}$ were estimated from the study of Griffis et al. (*31*). Again, the model predictions compared favorably with the data. The parameters k_{max} and $m_{1/2}$, which had been estimated from the elimination phase of the study by Griffis et al. (*31*), were valid in predicting burdens in both the accumulation and elimination phases of all studies. However, the model tended to underestimate burdens at later stages of the experiments (1 year after termination of exposure).

Discussion

As noted in the introduction, investigators have long sought to develop an accurate model for the retention of insoluble particles in the lung. Indeed, many retention models have been published for the rat lung which, as shown in Table 3, contain varying numbers of compartments (1–7) and clearance-related parameters (3–13). As computational power and speed have improved, complex physiologically based models have become increasingly popular because they

offer avenues for explaining the behaviors observed. However, in the absence of particularly rich and varied data resources, questions of accuracy tend to plague such highly parameterized models. Thus, when data are sparse, simple empirical models offer advantages for the accurate prediction of kinetic behavior, even if the reasons for such behavior remain elusive. Certainly our model (defined by Equations 2 and 3) falls into this latter category because it contains only a single compartment and two clearance-related parameters, k_{max} and $m_{1/2}$. We previously showed that k_{max} and $m_{1/2}$ are easily estimated and evaluated by applying simple linear regression techniques to modest amounts of clearance data (*27*). We regard this as a particular strength given the sparseness of data that can currently be used to assess the fit of any dust-retention model.

Despite its simplicity, our model reasonably described the mass-dependent behavior of lung burdens in rats exposed to four dusts (Sb_2O_3, PTT, CB, and DEP) over a wide range of particle sizes and experimental protocols. Thus, we conclude that MM-like kinetics captured the essential features of saturable AM-mediated clearance, which largely governed both the accumulation and elimination of lung burdens in inhalation studies involving insoluble dusts.

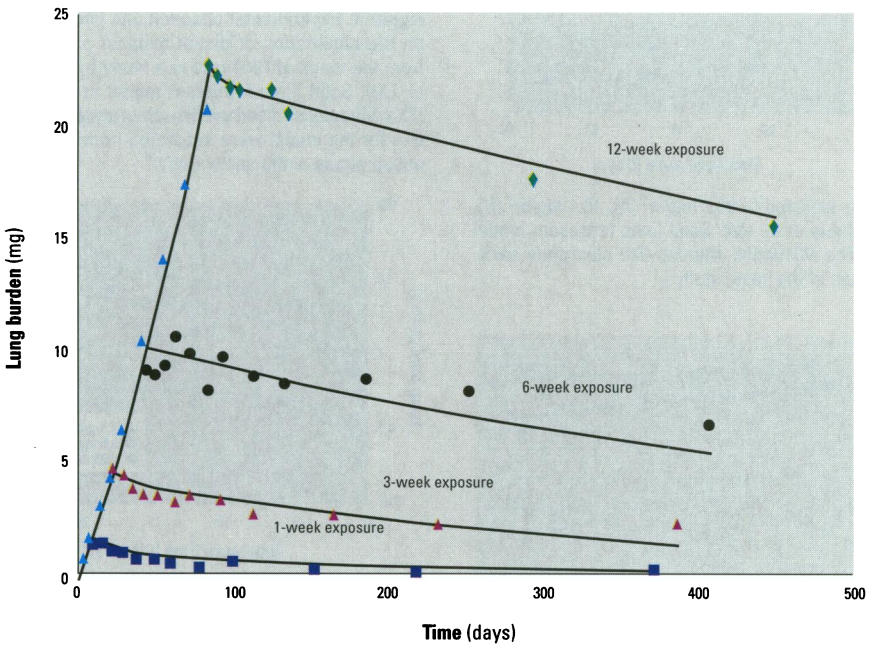

Figure 9. Experimental observations (symbols) on the accumulation and elimination of DEP in the lungs of F344 male rats in a study by Strom et al. (*5*). Solid lines represent model predictions. [The Michaelis–Menten-like clearance parameters for the model were estimated from the elimination phase of the study by Griffis et al. (*31*).]

Table 3. Comparison of models used to simulate accumulation and clearance of dusts in the rat lung

Retention model proposed by	Number of compartments[a]	No. of parameters[a,b]	Dust retention data used to validate the model
This study	1	2	DEP, PTT, CB, Sb_2O_3
Katsnelson et al. (*23,25*)	6	12–13	SiO_2, TiO_2
Smith (*12*)	5	7	SiO_2
Stöber et al. (*10,21*)	7	8–13	DEP, PTT, CB
Strom et al. (*5,6,13*)	3–4	3–6	DEP, CB
Vacek et al. (*22*)	2[c]	3[c]	SiO_2
Vincent et al. (*8*)	3–4	4–5	Asbestos fibers
Yu et al. (*45*)	1	4	Asbestos fibers
Yu et al. (*46*)	2	9	DEP

Abbreviations: DEP, diesel exhaust particulate; PTT, photocopy test toner; CB, carbon black; Sb_2O_3, antimony trioxide; SiO_2, crystalline silica; TiO_2, titanium dioxide.
[a]Excluding the lymphatic compartment.
[b]Excluding parameters associated with particle deposition.
[c]The overall model suggested by Vacek et al. (*22*).

During the accumulation phase of inhalation experiments, our model relies upon an empirically derived constant (E_A) to account for the efficiency of particle deposition in the lung. The ranges of E_A that we obtained were generally consistent with published values obtained from rats exposed to a variety of aerosols, including aluminosilicate (*4,36,37*), DEP (*38,39*) [note that 66% of lung deposition was assumed to be in the alveolar region (*39*)], Ga_2O_3 (*4,40,41*), PuO_2 (*42*), U_3O_8 and UO_2 (*43*), and an ^{198}Au labeled aerosol (*44*). We included monodisperse particles, characterized by their aerodynamic diameters (*36,37*) as well as polydisperse aerosols characterized by their MMADs (*4,38–43*). As shown in Figure 10, these published values of E_A displayed a decreasing trend with increasing size from about 20% for 0.1-μm particles to about 1% for 7-μm particles. All fits produced values of E_A within the range of those observed from experimental studies, with the exception of the fit of our model to the data from Strom et al. (*5*), where the estimated values of E_A ranged from 28 to 30% for DEP particles, with an MMAD of 0.19 μm. These values, however, were very similar to those reported by Strom et al. (26.5–38%) (*5,6*) and Stöber et al. (28.3%) (*10*). Thus, we conclude that the values of E_A that we assigned to the various experimental groups should not have unduly influenced the behavior of our model predictions.

The validity of MM-like kinetic models is considered from three perspectives. First, reasonable agreement is to be expected between model predictions and observed lung burdens when the same data are used to estimate the clearance-related parameters and also to evaluate goodness of fit (as for Sb_2O_3 in Fig. 1, for PTT in Fig. 3,4, for CB in Fig. 6, and for DEP in Fig. 7). Second, all of the pairs of k_{max} and $m_{1/2}$ that were used to define clearance rate coefficients for the four dusts were obtained from the elimination phases of inhalation experiments after exposure had been terminated. Yet, we observed excellent agreement between observed lung burdens and model predictions during the accumulation phases of all experiments as well (Fig. 1,5,8,9). Third, more validity can be given to a model in which parameters estimated from one study successfully predict lung burdens from another, as was the case for PTT (Fig.

5) and DEP (Fig. 8,9). Such agreement of model predictions across studies for two different dusts strengthens the notion that MM-like clearance behavior may be generally applicable to insoluble particles.

We suspect that the parsimony achieved by applying MM-like kinetics to alveolar clearance relates to its underlying biological plausibility. Table 4 compares the main characteristics of traditional MM enzyme kinetics to the analogous process that we postulate to be operative in the lung. The key feature leading to the mathematical forms of both relationships rests in the inherent feedback systems, which are related to dissociation of enzyme-substrate complex on the one hand and to redistribution of phagocytized particles (associated with the deaths of AMs) on the other. Although the concept of particle redistribution has long been recognized and has often been included in retention models (*10,20,22–25*), we are apparently the first to recognize the parallel between redistribution and dissociation of the enzyme-substrate process and, thereby, to justify the mathematical simplicity of MM kinetics in this context.

The MM-like kinetics that underlie our model also lend substance to the notion of lung overload, where it is postulated that the clearance rate slows only after some critical threshold burden has been exceeded. From Equation 1 it is easily shown that when $m << m_{1/2}$, linear (or first-order) kinetics prevail and the alveolar clearance rate coefficient k is approximately equal to k_{max}. Thus, the threshold burden is defined by the condition where $m << m_{1/2}$. Previously (*27*), we estimated threshold burdens of 0.11, 0.16, 0.40, and 0.46 mg for Sb_2O_3, PTT, DEP, and polyvinyl chloride powder, respectively (whose values of $m_{1/2}$ were estimated to be 0.69, 0.97, 2.49, and 2.90 mg, respectively). This implies that lung overload occurs when the lung burden exceeds about 16% of $m_{1/2}$. The good fits of all experimental data to our model underscore the point that dust overloading is probably a manifestation of nonlinear kinetics of the MM type according to the relationship given in Equation 1.

Our model can also be interpreted in the context of particle sequestration, where it has been postulated that clearance stops completely for some portion of the lung burden (*15,16*). Yu et al. (*35*) argued that particle sequestration can be explained in practice by the effect of slowed alveolar clearance rather than a complete breakdown of the clearance process, given that animals have a finite lifetime. Our model offers a similar explanation in the sense that alveolar clearance, under Equation 1, is mass-dependent and, in the extreme, will

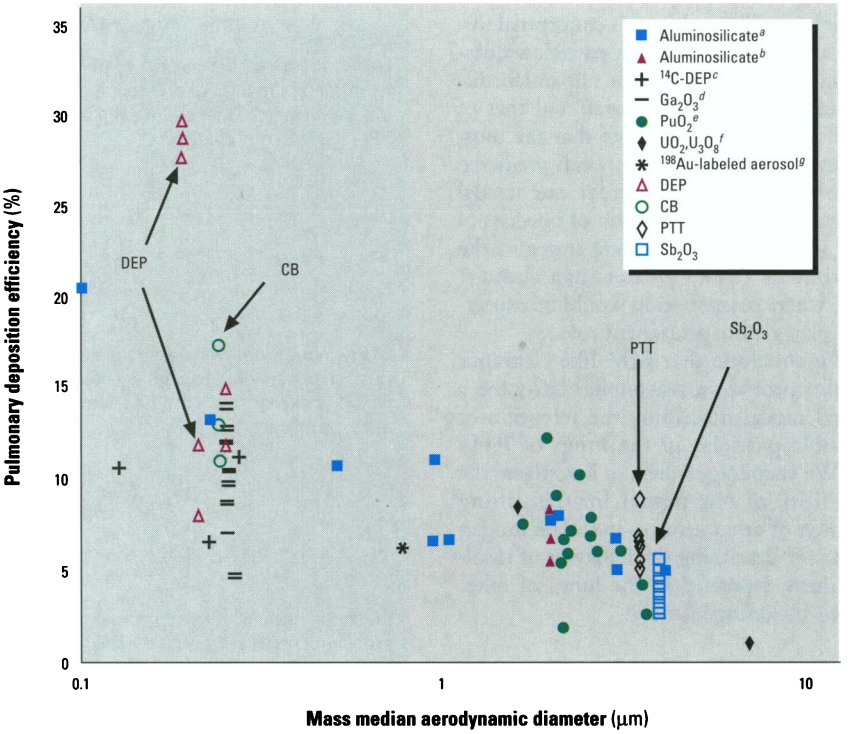

Figure 10. Model-fitted (open symbols) and other published empirical values of pulmonary deposition efficiency of various particles in rats. MMAD, mass median aerodynamic diameter; DEP, diesel exhaust particulate; CB, carbon black; PTT, photocopy test toner.
[a]MMAD = 0.1–4.18 μm [data from Raabe et al. (*36,37*)].
[b]MMAD = 2 μm [data from Wolff et al. (*4*)].
[c]MMAD = 0.125 μm [data from Chan et al. (*38*)] and MMAD = 0.23 and 0.27 μm [data from Dutcher et al. (*39*)].
[d]MMAD = 0.25 μm [data from Wolff et al. (*4,40,41*)].
[e]MMAD = 1.7–3.6 μm [data from Craig and Buschbom (*42*)].
[f]MMAD = 7.02 μm (U_3O_8) and MMAD = 1.62 μm (UO_2) [data from Stokinger et al. (*43*)].
[g]MMAD = 0.78 μm [data from McMahon et al. (*44*)].

Table 4. Traditional Michaelis–Menten (MM) enzymatic kinetics versus the MM-like kinetics of alveolar clearance of dusts

Characteristics	Enzyme kinetics	MM-like kinetics of dusts
Two-stage process	Substrate	Free particles
	Enzyme	AM
	ES	Phagocytized particles
	Reaction product	Cleared particles
	Enzyme recyling	Continuous supply of AM from bone marrow
Feedback system	ES dissociation	Particle redistribution
Overall kinetics	Saturable, nonlinear	Saturable, nonlinear
Kinetic variable	v, rate of enzymatic reaction	k, alveolar clearance rate coefficient
Kinetic parameters	V_{max}, maximal rate of enzymatic reaction	k_{max}, maximal alveolar clearance rate coefficient
	k_m, substrate concentration at which the rate is half of V_{max}	$m_{1/2}$, lung burden at which the clearance is half of k_{max}
Kinetic equation	$v = \frac{V_{max} \cdot [S]}{k_m + [S]}$	$k = \frac{k_{max} \cdot m_{1/2}}{m_{1/2} + m}$
Pseudo-linear condition	$[S] << k_m$, leading to $v \cong (V_{max}/k_m) \cdot [S]$	$m << m_{1/2}$, leading to $k \cong k_{max}$
Parameter estimation	Linear regression, e.g., Lineweaver-Burk method	Linear regression, $T_{1/2} = \alpha + \beta \cdot m$, for $k_{max} = \ln(2)/\alpha$ and $m_{1/2} = \alpha/\beta$

Abbreviations: S, substrate; E, enzyme; AM, alveolar macrophages; ES, enzyme–substrate complex.

lead to radically diminished clearance rate coefficients. Thus, although conceptual differences remain in defining particle sequestration, in practice there is not much difference between our approach and that of Yu et al. (*35*). We also note that the most consistent bias observed between predicted and observed burdens under our model involved the underestimation of burdens of DEP (Fig. 9–11) during late stages of the experiments (times greater than about 1 year), where sequestration would be expected to play a more prominent role.

We conclude that MM-like clearance kinetics provide a reasonable basis for a general model describing the retention of insoluble particles in the lungs of F344 rats. We encourage others to investigate the suitability of our model for describing retention of other aerosols in the rat lung as well as for describing the behavior of insoluble dusts deposited in the lungs of other species, including humans.

APPENDIX A: Difference Equations for Numerical Integration

Let m_t represent the burden of particles in the lung at time t. Then, Equation 2 in the text can be rewritten in the form of difference equation by substituting m_t for m, Δt (a finite time interval) for dt, (m_{t+1} - m_t) (a finite mass difference between times at 't+1' and t) for dm, and $\frac{k_{max} \cdot m_{1/2}}{m + m_t}$ [from Equation 1 in the text] for k. This gives

$$\frac{m_{t+1} - m_t}{\Delta t} = V_t \cdot E_A \cdot x - \frac{k_{max} \cdot m_{1/2}}{m_{1/2} + m_t} \cdot m_t, \quad \text{(A1)}$$

where x represents the dust concentration.

Rearrangement of the above equation yields the difference equation, which can be applied during the period of exposure to dust, i.e., when deposition and clearance of particles are simultaneously operating, as follows:

$$m_{t+1} = m_t + V_t \cdot E_A \cdot x \cdot \Delta t - \frac{k_{max} \cdot m_{1/2}}{m_{1/2} + m_t} \cdot m_t \cdot \Delta t. \quad \text{(A2)}$$

When the animals are not exposed to dusts, either after daily exposure, during weekends (e.g., for an exposure regimen in which the animals are exposed 6 hr/day for 5 days/week), or after termination of exposure, the kinetic process described by Equation 3 in the text can be rewritten in difference form as

$$m_{t+1} = m_t - \frac{k_{max} \cdot m_{1/2}}{m_{1/2} + m_0} \cdot m_t \cdot \Delta t \quad \text{(A3)}$$

where m_0 represents initial postexposure lung burden immediately following the latest exposure. Note that a time step Δt of less than 24 hr was used for all analyses. This time step is much smaller than $1/k_{max} \cong 110$ days for the four dusts investigated.

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